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Reproductive factors and risk of melanoma: a population-based cohort study

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Keywords: melanoma, reproductive factors, hormones, prospective cohort study, menstrual cycle

Bullet points:

What's already known about this topic?

- Female sex hormones have been suggested to play a role in the etiology of cutaneous melanoma (CM) and some epidemiological studies suggest that estrogen increase the risk of CM.
- The association with endogenous sex hormones have been studied through various reproductive factors, and parity, age at menarche, age at first birth and length of ovulatory life have been found to be associated with the risk of CM.

What does this study add?

- In this large nationwide population-based study of the association between reproductive factors and CM risk with detailed exposure and confounder information, no reproductive factors were clearly associated with CM risk.
- Association between reproductive factors and histological subtypes and anatomical sites are scarcely described in the literature. We observed significant heterogeneity in the effect of length of ovulatory life on the risk of superficial spreading melanoma and nodular melanoma.

Summary

Background

The association between reproductive factors and risk of cutaneous melanoma (CM) is unclear. We investigated this issue in the Norwegian Women and Cancer (NOWAC) cohort study.

Objectives

To examine the association between the reproductive factors age at menarche, menstrual cycle length, parity, age at first and last birth, menopausal status, breastfeeding duration and length of ovulatory life and CM risk, overall and by histological subtypes and anatomical site

Methods

We followed 165,712 women aged 30-75 at inclusion from 1991-2007 to the end of 2015. Multivariable Cox regression was used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs).

Results

The mean age at cohort enrolment was 49 years. During a median follow-up of 18 years, 1,347 CM cases were identified. No reproductive factors were clearly associated with CM risk. When stratifying by histological subtype we observed significant heterogeneity (p = 0.01) in the effect of length of ovulatory life on the risk of superficial spreading melanoma (HR 1.02, 95% CI 1.01-1.04 per year increase) and nodular melanoma (HR 0.97, 95% CI 0.94-1.01 per year increase). When stratifying by

anatomical site, menopausal status (HR 0.54, 95% CI 0.31-0.92, postmenopausal compared to premenopausal) and menstrual cycle length (HR 1.07, 95% CI 1.01-1.13, per day increase) were associated with CM of the trunk, and significant heterogeneity between anatomical sites was observed for menopausal status (p = 0.04).

Conclusions

In this large population-based Norwegian cohort study, we did not find convincing evidence of an association between reproductive factors and risk of CM.

Introduction

The incidence of cutaneous melanoma (CM) is rising in Caucasian populations, despite recent improvements in prevention and diagnosis (1). In 2012 CM was estimated to account for 232,000 new cancer cases and 55,000 deaths worldwide (2).

Exposure to solar ultraviolet (UV) radiation and indoor tanning, having a fair skin complexion, presence of many nevi and freckles, light hair, and skin reaction to the sun are important CM risk factors(3, 4). Female sex hormones, both endogenous and exogenous, have also been suggested to play a role in the etiology of CM. The fact that a better CM prognosis is observed in females than in males, and that the incidence of CM is higher among women than men between the age of 20 and 45 years, but that an opposite trend is observed after the age of 50 suggests that female sex hormones might influence CM development and prognosis (5, 6). The association between female sex hormones and risk of CM is biologically plausible as both estrogen receptors α, β and the non-standard G protein-coupled estrogen receptor, as well as progesterone receptors, are found in CM tissue (7, 8). Some in vitro experiments suggested that estrogen might increase proliferation of

melanocytes and CM cells, while progesterone possibly acts as an anti-proliferative and pro-apoptotic agent (8-12).

A large Dutch case-control study found a strong detrimental effect of oral contraceptives (OC) and unopposed estrogen hormone therapy (HT) on CM risk (13). In a large cohort study from Norway on HT, estrogen was positively associated with CM risk, while progestin seemed to be protective of CM (14). However, a meta-analysis of epidemiological studies up to 2009 found no association between HT or OC and CM risk (15). The meta-analysis did, however, find age at first birth to be positively associated with CM risk (i.e. the older the age the higher the risk), and parity to be inversely associated with CM risk. Additionally, did and a large Swedish case-control study report both parity and early childbearing to be protective of CM, although personal UV exposure was not adjusted for (16). A large French cohort study from 2011 found late age at menarche, early natural menopause and shorter ovulatory life to be associated with lower risk of CM (17).

Overall, the association between female sex hormones and CM risk is still controversial, and there has been little focus on hormones in relation to CM histological subtypes and anatomical sites relating to the divergent pathways hypothesis (18). We studied the association between the reproductive factors age at menarche, menstrual cycle length, parity, age at first and last birth, menopausal status, breastfeeding duration and length of ovulatory life, and risk of CM overall and by histological subtype and anatomical site in a large nationwide population-based cohort.

Material and methods

Data source

The Norwegian Women and Cancer (NOWAC) cohort was established in 1991 as a large nationwide population-based cohort in Norway. Cohort characteristics of NOWAC have been described in detail elsewhere (19). Briefly, invitation letters were sent to random samples of in total 327,476 women aged 30-75 in 1991-2007 with a response rate of 53%. Women who answered the baseline questionnaire were sent follow-up questionnaires every 4-6 years (response 80% for the second and 79% for third questionnaire). Follow-up was evaluated by linkage to the Cancer Registry of Norway for information on cancer diagnosis and vital status.

In total, we included 172,478 women answering the baseline questionnaire. We excluded women with a cancer diagnosis other than non-melanoma skin cancer prior to inclusion (n=6,694) and date of death or emigration prior to inclusion (n=72), resulting in a final sample size of n=165,712 (Fig. 1).

Exposure and outcome

Participants were asked about their reproductive history in the baseline and follow-up questionnaires. The exposures of interest were age at menarche (≤11, 12, 13, 14 or ≥15 years), menstrual cycle length during midlife defined as number of days between the first day of menstruating in two consecutive cycles (<25, 25-30 or >30 days), parity (including stillbirths) (0, 1, 2, 3 or ≥4 children), age at first birth (<22, 22-23, 24-26 or ≥27 years), age at last birth (<26, 26-28, 29-32 or ≥33 years), total breastfeeding duration (0, 1-4, 5-9, 10-16 or ≥17 months) and menopausal status (premenopausal, postmenopausal), defined based on the question “How old were you when the menstruation ceased?”. Women with missing information on age at menopause (37%) were coded as menopausal at age 53, which is the cutoff used in the Million Women Study (20) and the

validity has been demonstrated in a previous NOWAC publication (21). Length of ovulatory life was calculated as the age difference between menopause and menarche subtracting 9 months for each pregnancy, and categorized according to quartiles.

The outcome was incident CM using the Cancer Registry of Norway modified version of the International Classification of Diseases 7th revision (ICD-7 codes 1900-1909). Anatomical site was defined as head/neck (190.0), trunk (190.1/190.7), upper limbs (190.2) and lower limbs (190.3/190.4). Histological subtype was defined using ICD-Oncology 3rd edition codes (superficial spreading melanoma (SSM) = 8743.3 and nodular melanoma (NM) = 8721.3; other subtypes were too rare to be included).

Covariates

Region (latitudes 71°N – 58°N) of residential ambient UV exposure was categorized according to average number of hours of ambient residential UV exposure (low (northern Norway), medium-low (central Norway), medium (southwestern Norway), highest (southeastern Norway) (22). Birth cohort was categorized in 5-years intervals (<1940, 1940-1944, 1945-1949 and ≥1950). Body surface area (BSA) (m^2) was calculated using the DuBois and DuBois' equation ($weight^{0.4253}$ x $height^{0.7253}$ x 0.007184) and categorized according to quartiles (23). We categorized smoking as (never, past or current), education (≤10, 11-13 or >13 years) and marital status (married/partnered or not married/partnered) at cohort enrolment (baseline). Host pigmentation included untanned skin color (recorded on an 1 x 9 cm color scale graded from 1 (very fair) to 10 (very dark); categorized as dark (6-10), medium (4 and 5) or light (1-3)), hair color (black/dark brown, brown, blond/yellow or red) and number of asymmetric nevi >5 millimeters on legs (0, 1 or ≥2). Lifetime UV exposure until cohort enrolment included mean number of sunburns per year (0, ≤1, 1-2 or >2), mean number of weeks per year spent on sunbathing vacation (0, ≤1, 1-2, 2-3 or >3), and use of indoor tanning devices

(never, age at initiation <30 years or age at initiation ≥30 years). These were calculated according to Ghiasvand et al. (24, 25). None of the exposures were adjusted for use of OC or HT, as adjusting for this did not change the estimated associations.

Statistical analysis

Hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated by Cox regression using age as time scale and left truncated at age of first questionnaire. Diagnosis of CM was the event of interest. Women were censored at death, emigration, cancer diagnosis other than CM, except non-melanoma skin cancer, or end of follow-up (December 31, 2015), whichever occurred first.

Parity and menopausal status were analyzed as time-dependent variables. Age at last birth, duration of breastfeeding and length of ovulatory life were only analyzed among postmenopausal women, starting follow-up at age of menopause. When analyzing age at first and last birth only parous women were included.

All estimates were adjusted for residential ambient UV exposure, birth cohort, host pigmentation (hair color, skin color and large asymmetric nevi) and personal UV exposure (sunburns, bathing vacations and indoor tanning). Additional potential adjustment variables were selected based on simplified directed acyclic graphs for each exposure, and only variables that significantly improved the fit of the model were included.

When analyzing the association of reproductive factors by anatomical site only CM diagnosis at that specific site was analyzed as event, while CM in other sites were considered as censoring events. We did the same for histological subtypes. Heterogeneity between histological subtypes and anatomical sites was evaluated by contrast tests (26).

We performed sensitivity analysis, excluding women with very dark skin (grades 8-10). We also adjusted for birth cohort by stratification (stratified Cox-regression) instead of regular adjustment. In a subgroup of women with available information on use of OC we subtracted years of OC use from the length of ovulatory life measure.

The exposures of interest and covariates had a varying degree of missing values (0 – 70%), see Supplementary table 1 and 2 for details. To assess the influence of missing values we used multiple imputation with chained equations, assuming that the missing values are missing at random (27). The imputation model included the outcome and all exposures and adjustment variables. We imputed 70 data sets and the estimates and standard errors were combined using Rubin's rules (28).

All tests were two sided with a 5% statistical significance level. Proportionality was assessed with Schoenfeld residuals. All statistical analyses were performed using R version 3.3.3 (http://cran.r-project.org) and the R-package mice, version 2.46.0 was used for multiple imputation (29).

Ethics

NOWAC has been approved by the Regional Committees for Medical and Health Research and the Norwegian Data Inspectorate. All participants have given written consent.

Results

We followed 165,712 women from cohort enrolment in 1991-2007 to the end of 2015. The median follow-up was 18.1 years (range <1 to 24.7 years) during which 1,347 incident CM occurred. Characteristics of CM cases and non-cases are described in Table 1.

The risks of CM associated with reproductive factors are reported in Table 2. Menarche at age 12 was significantly associated with an increased risk of CM compared to age 13 (HR 1.18, 95% CI 1.02-1.38), but no trend emerged. Menstrual cycle length during midlife, parity, age at first birth, menopausal status, age at last birth and breastfeeding duration were not significantly associated with CM risk.

When separating CM into SSM and NM, significant heterogeneity was found for length of ovulatory life (p=0.01) (Table 3). Length of ovulatory life was significantly positively associated with SSM (HR 1.02, 95% CI 1.00-1.04, per year increase) and not associated with NM (HR 0.97, 95% CI 0.94-1.01).

Table 4 displays the anatomical site-specific results for the reproductive factors. Significant heterogeneity was observed for menopausal status (p=0.04). Postmenopausal women had a significantly lower risk of CM of the trunk (HR 0.54, 95% CI 0.31-0.92) as compared to premenopausal women. In addition, menstrual cycle length during midlife was associated with a significantly increased risk of CM of the trunk (HR 1.07, 95% CI 1.01-1.13, per year increase), but with no significant heterogeneity between sites (p=0.07).

As a sensitivity analysis we excluded women with very dark skin (n=2,491) and the results did not change substantially (data not shown). Additionally, when adjusting for birth cohort by stratification the results were similar as with regular adjustment (data not shown). When analyzing length of ovulatory life calculated as the age difference between menopause and menarche subtracting 9 months for each pregnancy *and* years of OC use in the subset of women with available OC information, the results were similar to the analyses with years of OC use not subtracted (data not shown). Multiple imputation generally resulted in very similar estimates to the main analyses (data not shown), but some discrepancies were observed. In particular, the association between menstrual cycle length and CM of the trunk was no longer statistically significant (HR 1.02, 95% CI 0.99-1.04 per year increase).

Discussion

In this nationwide population-based cohort we evaluated the association between several reproductive factors and CM risk. Our results suggest that reproductive factors are not associated with CM risk. However, we observed some heterogeneity between CM histological subtypes and between anatomical sites. Each year increase in length of ovulatory life was significantly associated with a 2% increased risk of SSM, and postmenopausal women were at significantly lower risk of CM of the trunk as compared to premenopausal women.

A number of epidemiological studies have evaluated the association between exogenous female sex hormones and CM, but the associations with use of menopausal hormone therapy (HT) and with oral contraceptives (OC) are still controversial. A large meta-analysis comprising six cohort studies and 19 case-control studies published up to 2009 found no significant associations between either HT or OC (15), and three newer studies found no association (30), an increased risk of HT estrogen and decreased risk of HT progestin (14) and a protective effect of HT/OC use (31), respectively.

Endogenous female sex hormones have been evaluated in a number of epidemiological studies through reproductive factors. The meta-analysis of studies published up to 2009 reported an increased risk of melanoma in women with late age at first birth, but no association with menopausal status, age at menopause, age at menarche, exams for fertility or parity (15). A newer meta-analysis comprising three case-control, three nested case-control and five cohort studies published up to 2014 reported a pooled relative risk of 1.47 (95% CI 1.07-2.02) comparing oldest to youngest age at first birth (32). Although there seem to be an association between age at first birth and CM risk, which is in contrast to our null finding, the hormonal mechanism is questionable as Kaae et al. found similar estimates for age at first birth in males and females in a large Danish study comprising 5,688 CM cases, and suggested life-style factors to be a more likely explanation (33).

In accordance with the meta-analysis of studies published up to 2009 we did not find a convincing association with age at menarche (15). However, Kvaskoff et al. reported a significantly reduced risk of CM in women with later age at menarche, and shorter length of ovulatory life (17). The latter is, however, in line with our non-significant association of 1% increase per 1 year increase in ovulatory life. Our measure of length of ovulatory life did not account for use of OC, however in the sensitivity analysis with years of OC use subtracted from length of ovulatory life, the result was similar.

The associations between reproductive factors and CM histology and site are scarcely described in the literature. Kvaskoff et al. reported mainly comparable estimates across CM histology (17). This is in contrast to our findings of opposite associations of length of ovulatory life for SSM and NM, but the number of cases within each histology in Kvaskoff et al. was very low and our estimates are close to 1 and only borderline significant for SMM. Kvaskoff et al. noted a significant heterogeneity for nulliparity between head/neck and trunk, although the individual estimates were not statistically significant. Our results indicate a similar opposite association for head/neck (HR 0.51, 95% CI 0.22-1.16) and trunk (HR 1.14, 0.95% CI 0.85-1.54), but with no significant heterogeneity (p=0.07). We found significant heterogeneity across CM sites for menopausal status, which was not observed in Kvaskoff et al. This heterogeneity may be due to residual confounding by age as CM on the trunk tend to occur at earlier ages, especially in women (34).

The association between sex hormones and risk of CM is biologically plausible, but the mechanisms through which they exert they effect are still largely unknown. *In-vitro* experiments have suggested that estrogen might increase proliferation of melanocytes and CM cells, while progesterone possibly acts as an anti-proliferative and pro-apoptotic agent, counteracting the stimulatory effect of estrogen (8-12), which might explain the large amount of null-findings in the literature. Another possible mechanism is through telomere length, where longer telomeres have been found to increase the risk of melanoma (35, 36). Estrogen upregulates telomerase in *in-vitro* experiments, and

longer ovulatory life and higher parity have been found to respectively increase and decrease telomere length (37-39).

The major strengths of this study are the representative, nationwide population-based prospective design with a follow-up of up to 25 years, the detailed covariate information and the accurate outcome information through linkage with high quality national registries. The design of the study allows for generalizability of findings to the whole country, and perhaps broader. The major limitation is that all covariates are self-reported, thus some misclassification is likely to have occurred, but it was most probably non-differential, since all the information was collected before CM diagnosis. We have not adjusted for multiple testing, but it is clear that none of the estimates in the sub analyses, nor the heterogeneity tests, would continue to be significant.

In conclusion, no reproductive factor was clearly associated with CM risk in this nationwide cohort study.

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Figure legends

Figure 1. Flowchart of study participants: The NOWAC Study.

Table 1. Baseline characteristics of the study participants and association with melanoma risk: The NOWAC Study.

	Incident melanoma	
	Yes N (%)	**No N (%)**
Age in years[a]	48 (43, 56)	49 (43, 56)
Birth cohort, n = 165 712		
< 1940	137 (10)	14 458 (9)
1940 – 1944	234 (17)	20 605 (13)
1945 – 1949	408 (30)	47 248 (29)
≥ 1950	568 (42)	82 054 (50)
Education, n = 157 008		
≤ 10 years	412 (32)	55 733 (36)
11-13 years	416 (33)	45 990 (30)
> 13 years	448 (35)	54 009 (35)
Marital status, n= 160 129		
Married/partnered	1 056 (81)	126 253 (79)
Not married/partnered	247 (19)	32 573 (21)
Smoking status, n= 164 914		
Never	576 (43)	57 772 (35)
Past	456 (34)	56 084 (34)
Current	305 (23)	49 721 (30)
Body surface area, 161 590		
Q1: < 1.65 m^2	282 (21)	39 933 (25)
Q2: 1.65 – 1.73 m^2	324 (25)	40 148 (25)
Q3: 1.74 – 1.83 m^2	385 (29)	40 000 (25)
Q4: ≥ 1.83 m^2	324 (25)	40 194 (25)
Hair color, n = 151 700		
Black/dark brown	137 (11)	26 231 (17)
Brown	382 (31)	60 404 (40)
Blond/yellow	623 (51)	59 193 (39)
Red	88 (7)	4 642 (3)
Skin color, n = 131 261		
Very dark/dark	189 (18)	28 325 (22)
Medium	379 (35)	49 181 (38)
Light	503 (47)	52 684 (40)
Total no. of asymmetrical nevi with diameter >5 mm on legs, n = 145 641		
0	891 (76)	127 617 (88)
1	126 11)	9 713 (7)
≥ 2	154 (13)	7 140 (5)
Residential ambient UV exposure n = 165 712		
Low (northern Norway)	144 (11)	35 788 (22)
Medium-low (central Norway)	161 (12)	18 426 (11)
Medium(southwestern Norway)	279 (21)	30 562 (19)
Highest (southeastern Norway)	763 (57)	79 589 (48)
Mean sunburns per year, n = 122 083		
0	90 (9)	17 744 (15)
≤ 1	612 (60)	74 975 (62)

> 1 – 2	230(22)	21 280 (18)
> 2	93 (9)	7 059 (6)
Mean weeks of sunbathing vacations per year, n = 130 723		
0	129 (12)	16 713 (13)
≤ 1	300 (28)	38 454 (30)
> 1 – 2	352 (33)	42 475 (33)
> 2 – 3	157 (15)	18 760 (14)
> 3	136 (13)	13 247 (10)
Indoor tanning, n = 131 135		
Never	381 (35)	45 129 (35)
Age at initiation < 30 years	160 (15)	20 790 (16)
Age at initiation ≥ 30 years	555 (51)	64 120 (49)

[a]Median (interquartile range.

Table 2. Reproductive factors and risk of melanoma: The NOWAC Study.

	No. of cases	Person years	HR[a] (95% CI)
Age at menarche, n = 162,881			
≤ 11 years	99	234,772	0.87 (0.70 – 1.09)
12 years	295	514,209	1.18 (1.02 – 1.38)
13 years	368	749,315	Ref.
14 years	326	665,886	0.97 (0.84 – 1.13)
≥ 15 years	236	483,364	0.94 (0.80 – 1.11)
Per year	1324		0.98 (0.94 - 1.02)
Menstrual cycle length during midlife[b], n = 47,880			
< 25 days	63	141,304	0.96 (0.74 – 1.26)
25 – 30 days	421	896,872	Ref.
> 30 days	20	49,273	0.86 (0.55 – 1.35)
Per day	504		1.01 (0.98 – 1.04)
Parity[c,i], n = 165,712			
0 children	127	248,316	Ref.
1 child	134	312,595	0.90 (0.70 – 1.14)
2 children	580	1,123,020	1.06 (0.88 – 1.29)
3 children	366	707,996	1.11 (0.91 – 1.37)
≥ 4 children	140	302,388	1.04 (0.81 – 1.33)
Per child	1347		1.03 (0.98 – 1.08)
Nulliparous[c,i], n = 165,712			
No	1220	2,445,998	Ref.
Yes	127	248,316	0.95 (0.79 – 1.14)
Age at first birth[c,f], n = 149,863			
< 22 years	373	805,770	Ref.
22 – 23 years	234	474,191	0.96 (0.81 – 1.13)
24 – 26 years	310	571,500	1.00 (0.86 – 1.17)
≥ 27 years	303	594,450	0.94 (0.80 – 1.11)
Per year	1220		0.99 (0.98 – 1.01)
Menopausal status[d,i], n = 165,712			
Pre	226	752,486	Ref.
Post	1,120	1,938,493	0.83 (0.62 – 1.13)
Age at last birth[c,h], n = 127,350			
< 26 years	235	406,030	Ref.
26 – 28 years	223	372,334	0.97 (0.81 – 1.17)
29 – 32 years	282	480,843	0.94 (0.79 – 1.12)
≥ 33 years	274	504,874	0.88 (0.73 – 1.06)
Per year	1013		0.99 (0.98 – 1.00)
Breastfeeding duration[e,g], n = 85,406			
0 months	49	77,782	1.17 (0.85 – 1.62)
1 – 4 months	101	213,166	0.93 (0.72 – 1.20)
5 – 9 months	145	279,902	Ref.
10 – 16 months	165	274,857	1.14 (0.91 – 1.42)
≥17 months	194	340,141	1.07 (0.86 – 1.33)
Per month	654		1.00 (0.99 – 1.01)
Length of ovulatory life[b,g], n = 142,611			
Q1: < 33.5 years	267	457,714	0.95 (0.80 – 1.12)

Q2: 33.5 – 36.5 years	268	531,090	Ref.
Q3: 36.6 – 38.4 years	224	378,113	0.99 (0.83 – 1.19)
Q4: ≥ 38.5 years	212	349,846	1.03 (0.86 – 1.24)
Per year	971		1.01 (0.99 – 1.02)

[a]Hazard ratios (HRs) and 95% confidence intervals (CIs) from Cox regression with age as the time scale (age adjusted) and adjusted for residential ambient ultraviolet (UV) exposure, birth cohort, host pigmentation (hair color, skin color and large asymmetric nevi) and UV exposure (sunburns, bathing vacations and solarium use). [b]Additionally adjusted for body surface area (BSA) and smoking. [c]Additionally adjusted for education. [d]Additionally adjusted for BSA, smoking and education. [e]Additionally adjusted for marital status and smoking. [f]Only in parous women. [g]Only in postmenopausal women. [h]Only in parous postmenopausal women. [i]Analysed as time-dependent.

Table 3. Reproductive factors and risk of melanoma by histological subtype: The NOWAC Study.

	Superficial spreading melanoma		Nodular melanoma		
	No. of cases	HR[a] (95% CI)	No. of cases	HR[a] (95% CI)	p for heterogeneity
Age at menarche, n = 162,881					
≤ 11 years	58	0.86 (0.65 – 1.16)	17	1.01 (0.59 – 1.75)	
12 years	190	1.30 (1.07 – 1.58)	38	1.02 (0.68 – 1.54)	
13 years	215	Ref.	55	Ref.	
14 years	199	1.03 (0.85 – 1.25)	43	0.85 (0.57 – 1.27)	
≥ 15 years	144	1.01 (0.81 – 1.24)	36	0.93 (0.61 – 1.42)	
Per year	806	0.98 (0.93 – 1.03)	189	0.96 (0.87 – 1.07)	0.76
Menstrual cycle length during midlife[b], n = 47,880					
< 25 days	35	0.86 (0.60 – 1.22)	10	1.01 (0.52 – 1.97)	
25 – 30 days	264	Ref.	62	Ref.	
> 30 days	14	0.96 (0.56 – 1.63)	4	1.20 (0.43 – 3.30)	
Per day	313	1.02 (0.99 – 1.07)	76	1.04 (0.96 – 1.13)	0.77
Parity[c,i], n = 165,712					
0 children	80	Ref.	19	Ref.	
1 child	84	0.88 (0.65 – 1.19)	10	0.46 (0.21 – 0.99)	
2 children	357	1.03 (0.81 – 1.31)	93	1.19 (0.73 – 1.96)	
3 children	208	1.02 (0.79 – 1.32)	48	0.98 (0.57 – 1.67)	
≥ 4 children	92	1.16 (0.86 – 1.58)	21	0.92 (0.49 – 1.74)	
Per child	821	1.04 (0.98 – 1.10)	191	1.02 (0.90 – 1.14)	0.73
Nulliparous[c,i], n = 165,712					
No	741	Ref.	172	Ref.	
Yes	80	0.98 (0.78 – 1.24)	19	1.00 (0.62 – 1.61)	0.95
Age at first birth[c,f], n = 149,863					
< 22 years	222	Ref.	54	Ref.	
22 – 23 years	152	1.05 (0.85 – 1.29)	30	0.89 (0.57 – 1.40)	
24 – 26 years	189	1.02 (0.83 – 1.25)	45	1.09 (0.72 – 1.65)	
≥ 27 years	178	0.92 (0.74 – 1.13)	43	1.03 (0.67 – 1.59)	

Per year	741	0.99 (0.98 – 1.01)	172	0.99 (0.96 – 1.03)	0.96
Menopausal status[d,i], n = 165,712					
Pre	164		26	Ref.	
Post	656	0.72 (0.49 – 1.06)	165	1.22 (0.54 – 2.78)	0.25
Age at last birth[c,h], n = 127,350					
< 26 years	136	Ref.	34	Ref.	
26 – 28 years	128	0.98 (0.77 – 1.25)	37	1.10 (0.69 - 1.77)	
29 – 32 years	158	0.93 (0.74 – 1.18)	44	1.01 (0.64 – 1.59)	
≥ 33 years	169	0.98 (0.77 – 1.24)	32	0.70 (0.42 – 1.16)	
Per year	591	1.00 (0.98 – 1.02)	147	0.98 (0.94 – 1.01)	0.24
Breastfeeding duration[e,g], n = 85,406					
0 months	29	1.28 (0.83 – 1.96)	8	1.15 (0.51 – 2.56)	
1 – 4 months	61	1.02 (0.73 – 1.43)	15	0.84 (0.44 – 1.60)	
5 – 9 months	79	Ref.	24	Ref.	
10 – 16 months	91	1.15 (0.85 – 1.55)	27	1.15 (0.66 – 1.99)	
≥17 months	110	1.11 (0.83 – 1.49)	28	0.98 (0.56 – 1.70)	
Per month	370	1.00 (0.99 – 1.01)	102	1.00 (0.98 – 1.01)	0.69
Length of ovulatory life[b,g], n = 142,611					
Q1: < 33.5 years	138	0.80 (0.63 – 1.01)	53	1.34 (0.87 – 2.08)	
Q2: 33.5 – 36.5 years	162	Ref.	35	Ref.	
Q3: 36.6 – 38.4 years	131	0.95 (0.75 – 1.19)	35	1.20 (0.75 – 1.93)	
Q4: ≥ 38.5 years	130	1.03 (0.81 – 1.30)	23	0.87 (0.51 – 1.49)	
Per year	561	1.02 (1.00 – 1.04)	146	0.97 (0.94 – 1.01)	0.01

[a]Hazard ratios (HRs) and 95% confidence intervals (CIs) from Cox regression with age as the time scale (age adjusted) and adjusted for residential ambient ultraviolet (UV) exposure, birth cohort, host pigmentation (hair color, skin color and large asymmetric nevi) and UV exposure (sunburns, bathing vacations and solarium use). [b]Additionally adjusted for body surface area (BSA) and smoking. [c]Additionally adjusted for education. [d]Additionally adjusted for BSA, smoking and education. [e]Additionally adjusted for marital status and smoking. [f]Only in parous women. [g]Only in postmenopausal women. [h]Only in parous postmenopausal women. [i]Analysed as time-dependent.

Table 4. Reproductive factors and risk of melanoma by anatomical site: The NOWAC Study.

	Head and neck		Upper limbs		Trunk		Lower limbs		
	No. of cases	HR (95% CI)	No. of cases	HR[a] (95% CI)	No. of cases	HR[a] (95% CI)	No. of cases	HR[a](95% CI)	p for heterogeneity
Age at menarche, n = 162,881									
≤ 11 years	6	0.54 (0.23 – 1.27)	18	0.94 (0.56 – 1.58)	37	1.08 (0.74 – 1.56)	35	0.81 (0.56 – 1.18)	
12 years	23	0.91 (0.54 – 1.53)	46	1.07 (0.73 – 1.56)	98	1.32 (1.00 – 1.73)	117	1.24 (0.97 – 1.59)	
13 years	38	Ref.	64	Ref.	110	Ref.	138	Ref.	
14 years	29	0.81 (0.50 – 1.31)	51	0.86 (0.60 – 1.25)	110	1.11 (0.85 – 1.44)	119	0.95 (0.75 – 1.22)	
≥ 15 years	16	0.56 (0.31 – 1.02)	37	0.81 (0.54 – 1.22)	85	1.16 (0.87 – 1.54)	88	0.96 (0.74 – 1.26)	
Per year	112	0.94 (0.82 – 1.07)	216	0.93 (0.85 – 1.03)	440	1.00 (0.94 – 1.07)	497	0.98 (0.92 – 1.04)	0.63
Menstrual cycle length during midlife[b], n = 47,880									
< 25 days	8	2.01 (0.91 – 4.44)	7	0.66 (0.30 – 1.43)	14	0.65 (0.37 – 1.13)	29	1.12 (0.75 – 1.66)	
25 – 30 days	26	Ref.	70	Ref.	138	Ref.	167	Ref.	
> 30 days	0		5	1.31 (0.53 – 3.24)	10	1.33 (0.70 – 2.52)	4	0.43 (0.16 – 1.17)	
Per day	34	0.94 (0.85 – 1.03)	82	1.03 (0.95 – 1.11)	162	1.07 (1.01 – 1.13)	200	0.99 (0.94 – 1.03)	0.07
Parity[c,i], n = 165,712									
0 children	6	Ref.	17	Ref.	50	Ref.	46	Ref.	
1 child	11	1.74 (0.64 – 4.73)	21	1.11 (0.59 – 2.11)	47	0.78 (0.52 – 1.16)	48	0.87 (0.58 – 1.30)	
2 children	45	1.93 (0.82 – 4.53)	96	1.38 (0.82 – 2.31)	194	0.89 (0.65 – 1.22)	218	1.08 (0.78 – 1.48)	
3 children	33	2.06 (0.86 – 4.95)	62	1.36 (0.79 – 2.34)	108	0.85 (0.60 – 1.19)	150	1.26 (0.91 – 1.77)	
≥ 4 children	17	2.11 (0.82 – 5.42)	24	1.13 (0.60 – 2.12)	50	1.02 (0.68 – 1.52)	45	0.98 (0.64 – 1.49)	
Per child	112	1.08 (0.94 – 1.25)	220	1.03 (0.93 – 1.15)	449	1.02 (0.94 – 1.11)	507	1.04 (0.96 – 1.12)	0.92
Nulliparous[c,i], n = 165,712									
No	106	Ref.	203	Ref.	399	Ref.	461	Ref.	
Yes	6	0.51 (0.22 – 1.16)	17	0.77 (0.47 – 1.26)	50	1.14 (0.85 – 1.54)	46	0.92 (0.68 – 1.25)	0.22
Age at first birth[c,f], n = 149,863									
< 22 years	35	Ref.	48	Ref.	128	Ref.	145	Ref.	
22 – 23 years	17	0.67 (0.37 – 1.20)	53	1.62 (1.09 – 2.40)	66	0.81 (0.60 – 1.09)	90	0.97 (0.74 – 1.26)	
24 – 26 years	28	0.84 (0.50 – 1.41)	51	1.21 (0.80 – 1.82)	103	1.00 (0.77 – 1.32)	117	1.00 (0.77 – 1.29)	
≥ 27 years	26	0.73 (0.42 – 1.27)	51	1.26 (0.76 – 1.78)	102	0.97 (0.73 – 1.28)	109	0.90 (0.69 – 1.17)	
Per year	106	0.98 (0.94 – 1.03)		1.00 (0.96 – 1.03)	399	1.00 (0.97 – 1.02)	461	0.99 (0.96 – 1.01)	0.86
Menopausal status[d,i], n = 165,712									

Pre	16	Ref.	22	Ref.	76	Ref.	97	Ref.	
Post	96	0.36 (0.10 – 1.26)	198	1.70 (0.77 – 3.78)	373	0.54 (0.31 – 0.92)	409	1.08 (0.68 – 1.71)	0.04
Age at last birth[c,h], n = 127,350									
< 26 years	23	Ref.	37	Ref.	79	Ref.	87	Ref.	
26 – 28 years	14	0.57 (0.29 – 1.11)	41	1.09 (0.69 – 1.70)	73	0.98 (0.71 – 1.35)	83	1.00 (0.74 – 1.35)	
29 – 32 years	31	0.87 (0.50 – 1.51)	52	1.00 (0.65 – 1.54)	91	0.96 (0.71 – 1.31)	101	0.95 (0.71 – 1.27)	
≥ 33 years	23	0.54 (0.30 – 0.99)	54	0.94 (0.60 – 1.45)	89	0.95 (0.69 – 1.30)	99	0.93 (0.69 – 1.26)	
Per year	91	0.97 (0.93 – 1.02)	184	0.98 (0.95 – 1.01)	332	1.00 (0.98 – 1.02)	370	0.99 (0.97 – 1.01)	0.74
Breastfeeding duration[e,g], n = 85,406									
0 months	8	1.78 (0.75 – 4.20)	9	1.09 (0.52 – 2.30)	11	0.83 (0.43 – 1.61)	19	1.35 (0.79 – 2.29)	
1 – 4 months	14	1.31 (0.63 – 2.73)	17	0.79 (0.43 – 1.43)	34	0.98 (0.63 – 1.53)	35	0.94 (0.61 – 1.45)	
5 – 9 months	15	Ref.	30	Ref.	45	Ref.	49	Ref.	
10 – 16 months	9	0.57 (0.25 – 1.31)	31	1.01 (0.61 – 1.66)	55	1.24 (0.84 – 1.85)	62	1.27 (0.87 – 1.85)	
≥17 months	20	0.92 (0.47 – 1.81)	39	0.98 (0.60 – 1.58)	63	1.16 (0.79 – 1.71)	64	1.09 (0.75 – 1.59)	
Per month	66	0.98 (0.96 – 1.01)	126	1.00 (0.98 – 1.01)	208	1.01 (1.00 – 1.02)	229	1.00 (0.99 – 1.01)	0.29
Length of ovulatory life[b,g], n = 142,611									
Q1: < 33.5 years	22	1.10 (0.62 – 1.95)	42	0.80 (0.52 – 1.22)	78	0.82 (0.60 – 1.11)	111	1.07 (0.81 – 1.42)	
Q2: 33.5 – 36.5 years	26	Ref.	47	Ref.	94	Ref.	96	Ref.	
Q3: 36.6 – 38.4 years	19	1.13 (0.61 – 2.10)	43	1.17 (0.77 – 1.77)	65	0.78 (0.60 – 1.08)	82	1.00 (0.74 – 1.35)	
Q4: ≥ 38.5 years	16	1.12 (0.58 – 2.16)	38	1.16 (0.75 – 1.80)	81	1.06 (0.78 – 1.44)	71	0.94 (0.68 – 1.28)	
Per year	83	0.98 (0.94 – 1.03)	170	1.03 (0.99 – 1.07)	318	1.02 (0.99 – 1.05)	360	1.00 (0.97 – 1.02)	0.32

[a]Hazard ratios (HRs) and 95% confidence intervals (CIs) from Cox regression with age as the time scale (age adjusted) and adjusted for residential ambient ultraviolet (UV) exposure, birth cohort, host pigmentation (hair color, skin color and large asymmetric nevi) and UV exposure (sunburns, bathing vacations and solarium use). [b]Additionally adjusted for body surface area (BSA) and smoking. [c]Additionally adjusted for education. [d]Additionally adjusted for BSA, smoking and education. [e]Additionally adjusted for marital status and smoking. [f]Only in parous women. [g]Only in postmenopausal women. [h]Only in parous postmenopausal women. [i]Analysed as time-dependent.